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The Transfer of Antibody Formation by Means of a Polymorphonuclear Exudate

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In earlier experiments in hyperimmunised animals it was demonstrated that by freezing and thawing proteins could be extracted from the repeatedly washed cells of a polymorphonuclear exudate, which became bound with the specific antigen. On making an electrophoretic study of the rate of movement at different pH, it was seen that the character of these proteins was not identical with the serum antibodies. Following immunisation with a single dose of the antigen, antibodies were demonstrated in polymorphonuclear cells for only a short period after immunisation (Šterzl 1952, 1954).

In the latest work the presence of developing antibodies in tissues is determined by means of their transfer to non-immunised recipients which do not themselves react to an antigen by antibody formation (animals irradiated by X-ray, five-day-old rabbits — Šterzl 1955). In the present work the same method is used for carrying out a revision of earlier results on the basis of which it was assumed that antibodies are also formed in the cells of a polymorphonuclear exudate.

Materials and Method

The polymorhonuclear exudate was prepared by filling normal and immunised rabbits with 300 — 400 ml. physiological saline administered intraperitoneally. After 4—5 hours the exudate is drawn off and sedimentation of the cells is carried out by centrifuging at 500 g. The cellular sediment is washed three times in gelatinous physiological saline. During washing the number of cells is adjusted to approximately 20—30,000/μl., according to the degree of turbidity, and is then determined exactly by counting in a Bürker chamber. In the case of every exudate a smear is taken and a differential count made from 100 cells. Polymorphonuclear cells and typical lymphocytes are determined precisely; the other cells of the peritoneal exudate are grouped together as macrophages, in view of the difficulty of precise differentiation. After four hours' peritoneal filling in rabbits, the cells in the exudate other than polymorphonuclear cells average only 5—20 %. Adult rabbits weighing 2—3 kg. were used as donors; these were immunised by the intravenous route with *Salmonella paratyphi B* inactivated by heating for one hour at 70 °C. The number of doses and the intervals between the doses are given in the text to figures. In experiments in which cells were isolated from non-immunised animals and mixed with the antigen in vitro, the same antigen *(S. paratyphi B)* was used. The amount of spleen cells used in the experiment and the amount of antigen added to the cells in vitro are the same as in a previous communication (Šterzl 1957). These data are given in greater detail in the text relating to the individual figures.

The serum used for adsorption on to the cells of the peritoneal exudate and for experiments with the passive transfer of antibodies to young rabbits was rabbit serum obtained by immunisation with the same strain of *S. paratyphi B* as that used as the antigen. The titre of the serum was 1 : 400, i. e. it was not lower than the titre of antibodies in the serum of any of the donors from whose peritoneal exudate cells were obtained.

Washed cells, concentrated to the given number, were transferred by the intraperitoneal route to ve-day-old rabbits from which blood was collected by cardiac puncture. The collection intervals are
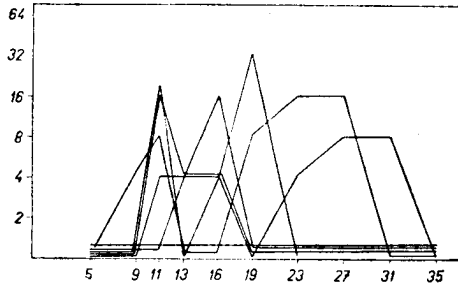
shown in the various figures concerned. The exact method for determining antibodies in young rabbits has already been described (Šterzl 1955). The agglutinations were placed in a refrigerator and the results read off after four and six days.

Results

Transfer of Cells of Polymorphonuclear Exudate Obtained at Various Intervals from Rabbits Immunised with One Dose of Antigen

Altogether 11 experiments were carried out in this series, transfer being made to 57 young rabbits from 11 litters. Rabbits were immunised with 1 ml. bacterial antigen by the intravenous route and peritoneal exudate produced at various intervals following immunisation. A different donor was used for every experiment.

Fig. 1. Formation of antibodies following transfer to young rabbits by means of cells of peritoneal exudate from donor (rabbit) immunised with single dose of antigen (10^8 micro-organisms). x: days after immunisation when peritoneal exudate was produced and cells obtained. Every recipient is denoted by one point. y: antibody values. The highest antibody titres reached during the ten days following transfer are always given. Recordings were made only from animals which survived and in which all collections of blood were made.

The exudates were prepared in the rabbits 2, 3, 4, 5, 6, 7 and 8 days after commencing immunisation. A survey of the results is given in fig. 1. It is seen that a positive demonstration of antibodies was obtained by the transfer of cells of peritoneal exudate isolated from rabbits four and five days after immunisation. The course of antibody formation following the transfer of cells from a rabbit four days after immunisation is shown in fig. 2. On comparing these results with results obtained from the transfer of spleen cells (Šterzl 1955, 1957) it is seen that in the present experiments with the transfer of cells of polymorphonuclear exudate antibody formation is less regular and very restricted as regards time.

Transfer of Cells of Polymorphonuclear Exudate Obtained from Repeatedly Immunised and Revaccinated Animals

In four experiments, rabbits which had been repeatedly immunised a number of times were used as donors. Transfer was made to 32 young rabbits. Fig. 3 gives an example of a transfer of cells from a revaccinated animal; fig. 4 shows the result of a transfer of cells from a repeatedly and intensively immunised donor. The doses are given in the appended text. As compared with immunisation with a single dose, a marked increase in the intensity of antibody formation in the recipients was observed, which was also of longer duration and more standard in character. The intensive and uniform formation of antibodies following the transfer of cells from a repeatedly and intensively immunised donor is particularly striking. As compared with the cell counts in the peritoneal exudate of animals immunised once and several times, it is seen that the intensive antibody formation observed in these last experiments cannot be explained by different percentual proportion of the various types of cells in the peritoneal exudate.
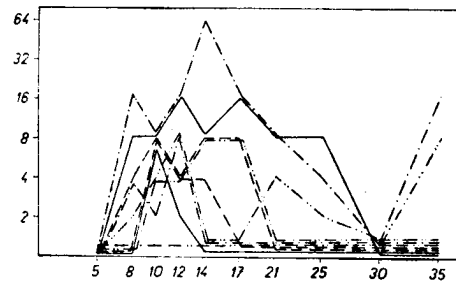
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Fig. 2.

Fig. 3.

Fig. 2. Normal rabbit immunised with 1 ml. (10^8micro-organisms) four days before formation of perito-neal exudate from 400 ml. physiological saline. Exudate drawn off after five hours by puncture and centrifuged. Exudate fluid, freed from cells, lyophilized (120 ml.) and dissolved in 6 ml. Agglutination titre of concentrated exudate fluid 1 : 16, rabbit serum 1:64. Cells washed and injected intraperitoneally in young rabbits in amounts of 1 ml. (228 × 10^6 cells). Extraction of cells carried out by freezing and thawing; agglutination reaction of extract negative. Cell count: polymorphonuclears 82, lymphocytes 17, macrophages 1. x: age of rabbits in days, y: titre of antibodies.

Fig. 3. Rabbits Nos. 801, 802, 803, 804, born 7. 12. 1954 and injected on fifth day of life with nucleo-protein fractions. Revaccinated on 12. 4. 1955 with 1 ml *Salmonella paratyphi B*, intravenously (10^7 micro-organisms), on 9. 5. 1955 with 1 ml. (2 × 10^9 micro-organisms). On 10. 5. 1955 filling of rabbits with physiological saline carried out; after washing, cells injected in amounts of 2 ml. (40 × 10^6 micro-organ-isms) in young rabbits. Cell count in exudates:
Rabbit No. 801: polymorphonuclears 98, lymphocytes 6, macrophages 6,
No. 802: polymorphonuclears 91, lymphocytes 9, macrophages 0,
No. 803: polymorphonuclears 90, lymphocytes 8, macrophages 2,
No. 804: polymorphonuclears 82, lymphocytes 8, macrophages 10.
Young rabbits Nos. 201 and 202 injected with 1 ml. leucocytes from donor No. 801 — full lines.
Young rabbits Nos. 203 and 204 injected with leucycytes from donor No. 803 — dashed line.
Young rabbits Nos. 205 and 206 injected with leucocytes from donor No. 804 — dotted line.
Young rabbits Nos. 207 and 208 injected with leucocytes from donor No. 802 — dash and two dots.
Control without injection — two dashes and two dots. x: age of rabbits in days; y: titre of antibodies.
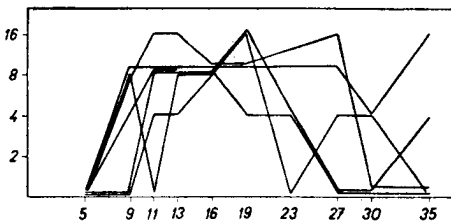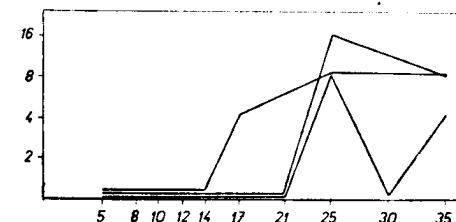

Fig. 4.

Fig. 5.

Fig 4. Rabbit No. 42, immunised from 30. 9. 1955 to 30. 1. 1956 three times weekly, first with seven doses of a suspension of 10^8 micro-organisms/ml., then with 20 doses of 2 × 10^9 micro-organisms/ml. Three days after last injection peritoneal exudate produced by a filling of 400 ml. physiological saline. After five hours the exudate was drawn off and the cells centrifuged. The supernatant fluid (80 ml.) was dried by lyophilization and dissolved in 5 ml. distilled water and dialyzed against physiological saline. Agglutination in the concentrated fluid was negative, 1 ml. of a washed suspension of cells (24 × 10^6 cells) injected intraperitoneally in five-day-old rabbits. Differential count: polymorphonuclears 82, lympho-cytes 6, macrophages 12. x: age of young rabbit and time of blood collection in days.y: titre of antibodies in blood of young rabbits.

Fig. 5. Normal rabbit filled with 500 ml. physiological saline. Resultant exudate washed five times in physiological saline. 228 × 10^5 cells/ml. After washing the cells were mixed with the antigen (1 cell of exudate to 2 micro-organisms of *S. paratyphi B*. The mixture was injected intraperitoneally in young abbits. x: age of young rabbit and time of blood collection in days. y: titre of antibodies in blood of young rabbits.

Transfer of Cells of Polymorphonuclear Exudate of Non-immunised Animals after Mixing with Antigen in vitro

Six experiments were carried out in this series, with transfer to 40 young animals. These experiments were based on the experience that if cells which are capable of changing their metabolism (e. g. spleen cells) are mixed with the antigen in vitro, they are able to form antibodies on being transferred intraperitoneally to young animals (Šterzl 1957). The cells of peritoneal exudate from normal animals were therefore isolated, mixed with the antigen and transferred to young rabbits by the intraperitoneal route. In none of these experiments was antibody formation observed in the first period following the transfer. The recording from one group (fig. 5) shows that antibodies cannot be demonstrated until the young animals are themselves able to react actively to an antigen by the formation of antibodies.

Transfer of Leucocytes from Non-immunised Animals, Incubated in vitro with Immune Serum

An attempt was made in earlier experiments (Šterzl 1952) to ascertain whether the transfer of antibody formation by means of cells of polymorphonuclear exudate is true antibody formation, or whether serum antibodies are only adsorbed on to the cells. In the present experiments, in which the transfer of cells was used to demonstrate antibody formation, the control experiments were carried out by the same method.
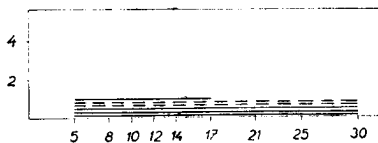
Fig. 6. Normal rabbit filled with 450 ml. physiological saline; exudate drawn off after five hours. Differential count: polymorphonuclears 86, lymphocytes 10, macrophages 4. After washing. 183 × 10⁵ cells/ ml. Cells transferred to two young rabbits — dashed lines. Remainder centrifuged and suspended in the same volume of immune serum against *S. paratyphi B* (titre 1: 400). Incubated with serum for 30 minutes at 37° C and 30 r. p. m. After incubation serum removed and titre of antibodies again determined (no change.) Cell sediment washed three times and suspended in original amount of fluid. Number of cells 176 × 10⁵/1 ml., 1 ml. cells injected intraperitoneally in young rabbits. Extract made from 2 ml. suspension of leucocytes by freezing and thawing; agglutination reaction of extract negative.

A known amount of serum antibodies was adsorbed on to the cells of a peritoneal exudate, the cells were washed three times in gelatinous physiological saline and transferred by the intraperitoneal route to young rabbits. In all, three such experiments were carried out on 25 young rabbits. In no case antibody was found, either in the young animals or in extracts of leucocytes. A detailed description of one of these experiments is given in fig. 6. In the same way, no decrease in the titre of antibodies (i. e. adsorption by the cells) was found following incubation of cells together with serum of a known titre. Negative demonstration of antibodies following transfer is easily understandable when antibodies were not found even by direct agglutination in an extract of cells. Passive transfer of serum of the given titre (1 : 400) was demonstrated in the serum of young rabbits if the injection was made with 1 ml. of concentrated serum and serum diluted in the proportion of 1 : 10. On injecting serum diluted in the proportion of 1:100, antibodies were not demonstrated serologically in the blood of young rabbits. It may therefore be assumed that if a slight amount of antibodies remains adsorbed on to the polymorphonuclear leucocytes, it will not be possible to demonstrate these antibodies serologically in the blood of young rabbits following transfer.

There is also a possibility, however, that a serologically demonstrable amount of antibodies, adsorbed on to the cells, may become bound, during preparation of the extract by freezing and thawing, to some components of the cells and lose their

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serological effectiveness. In such a case, although they might be present, it would not be possible to demonstrate the antibodies. This eventuality was verified by mixing a centrifuged suspension of cells (30 × 10^6/ml.) obtained from rabbit exudate with the same amount of serum of the titre given above. Extraction of the cells was carried out by freezing and thawing immediately after mixing with the serum and after incubating at 37 °C for 30 minutes. No change occurred in the titre of antibodies in the serum, either after simple incubation (they were not adsorbed on to the cells) or on mixing the cells with antibodies and disrupting them. This shows that cell components are not bound with serum antibodies in such a way as to mask their serological activity.

From these control experiments it is concluded that the antibody formation ascertained in preceding experiments following the transfer of cells of a polymorpho-nuclear exudate to young rabbits is the expression of biological activity of the cells and does not represent a passive transfer of already formed antibodies, but is the outcome of an active process of antibody formation by the cells.

Discussion

The literature on the question of antibody formation and its association with different types of cells has already been reviewed in an earlier communication (Šterzl 1954, pp. 45—51). It was shown that a number of authors associate antibody formation only with certain particular types of cells. More recently, especially among Scandinavian authors (Bjorneboe, Gormsen and Lundquist 1947, Fagraeus 1948) and in the work of Ehrich et al. (1949) some authors have come to regard plasmatic cells as the main site of antibody formation (e. g. Coons et al. 1955, Forshter 1955). Other experimental results, however, provide evidence that further types of cells participate in the formation of antibodies (Girard and Murray 1954, Roberts and Dixon 1955, Sinkovics 1955, Stoner and Hale 1955).

Not only in theory, but also in the experimental work, very little attention is paid to the participation of phagocytic cells in antibody formation. Any such study is based on the assumption expressed by Ehrich, Harris and Mertens (1946) that the participation of phagocytosing cells consists merely in engulfing and digesting the antigen so as to prepare the way for the actually active cells, the lymphocytes and plasmocytes. Since it has been demonstrated, however (Walsh and Smith 1951, Roberts 1955) that antigen digested by phagocytes decreases the antibody reaction, it is concluded that this does not participate in antibody formation. Further proof is to be found in experiments (Ehrich et al. 1946, Roberts 1955) demonstrating that phagocytic cells which invade inflammation of the skin or peritoneum and are then injected with antigen, do not form antibodies. It may be assumed that mature phagocytic cells which invade artificially produced inflammation are not capable of antibody formation. This is also demonstrated in our experiments in which the cells of an exudate were isolated, mixed with antigen and administered to young rabbits. No formation of antibodies was demonstrated in any of these experiments. On the other hand, antibody transfer was demonstrated using the same types of exudate cells, when the cells were collected four and five days after immunisation. The author takes the view that these experiments confirm the assumption that the cells of mesenchymal tissue can change their metabolism if they come into contact with antigen, not in a mature state, but in the course of their development.

It is especially necessary to estimate whether the transfer of antibody formation to young rabbits is mediated by the polymorphonuclear cells or whether other types of cells contained in small amounts in the exudate are responsible. When making the transfer, amounts of 20—30 × 10^6 cells are used; this is the smallest amount found

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to be satisfactory in making a transfer of very effective spleen cells. It is an amount many times less than that used by Harris (1954), the smallest quantity used by whom is 150×10^6. Since the transfer of antibody formation is also directly dependent on the quantity of transferred cells, it is hardly likely that so small a percentage of lymphocytes and macrophages would participate in the antibody reaction. In order to form a definite conclusion, however, it would be necessary to follow up the morphological fate of the various types of cells transferred and to ascertain whether the proportion determined by the count in the smear does not change in the recipient through proliferation of one type of cell.

The above results again support the assumption that antibody formation is a metabolic change in various cells and tissues. Particular significance is attached to the metabolic change in the course of immunisation in cells such as polymorphonuclear cells, which participate directly and to a large extent in the defence processes of the organism.

Summary

An investigation was made of the possibility of transferring antibody formation from adult immunised rabbits by means of cells of a polymorphonuclear exudate to five-day-old rabbits. Following a single immunisation dose of antigen (10^8 microorganisms of *Salmonella paratyphi B*), antibody formation was transferred to young rabbits by means of the cells of a polymorphoumlear exudate only when the cells had been obtained from the exudate four days after immunisation at the earliest. Antibody formation in young animals, when produced by the cells, of a donor, immunised by a single dose, is of short duration and not standard in character. The cells of a polymorphonuclear exudate obtained from adult donors repeatedly immunised with several doses of antigen produce antibody formation in young animals which is more intense, of longer duration and of a more standard nature than that produced by the transfer of cells from donors immunised with a single dose of antigen. The cells of a polymorphonuclear exudate obtained from normal, non-immunised rabbits and mixed with the antigen in vitro, never form antibodies on being transferred intraperitoneally to young rabbits. Adsorption of antibodies on to polymorphonuclear leucocytes was not demonstrated, either by direct serological tests or by transfer of the cells to young rabbits. It is concluded that the cells of polymorphonuclear exudate also participate in immunity processes in the organism, but only those cells that have come into contact with the antigen in the course of their development, and not the mature cells of polymorphonuclear exudate.

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Перенесение образования антител клетками полиморфонуклеарного экссудата

Я. ШТЕРЦЛЬ

Резюме

В своей работе мы определяли, возможно ли с помощью клеток полиморфо-
нуклеарного экссудата перенести способность к образованию антител от взрос-
лых иммунизированных кроликов на 5-дневных крольчат. После одноразовой
иммунизирующей дозы антигена (10^8 микробов S. paratyphi B) удавалось пере-
дать крольчатам эту способность клетками полиморфонуклеарного экссудата
только в случае, если эти клетки были получены из экссудата не раньше 4 дней
после иммунизации. Способность к образованию антител, вызываемая у молодых
животных клетками донора, иммунизированного одноразовой дозой, оказы-
вается нестандартной и скоропреходящей. Клетки полиморфонуклеарного
экссудата, полученного от взрослых доноров, повторно иммунизировавшихся
несколькими дозами антигена, вызывают у молодых животных более интен-
сивное, более длительное и более стандартное образование антител, чем перенос
клеток от доноров, иммунизированных одной дозой антигена. Клетки поли-
морфонуклеарного экссудата, полученные от нормальных, не иммунизиро-
ванных кроликов и смешанные с антигеном in vitro, после переноса в полость
брюшины крольчатам ни в одном случае не вызывали образования антител.
Наличие адсорбции антител на полиморфонуклеарные лейкоциты не было нами
доказано ни путем прямых серологических тестов, ни путем переноса клеток
крольчатам. Из опытов мы делаем заключение, что и клетки полиморфонуклеар-
ного экссудата принимают участие в перестройке организма в направлении
иммунитета, но это бывают только те клетки, которые столкнулись с антигеном
в процессе своего развития, а не уже сформировавшиеся клетки полиморфо-
нуклеарного экссудата.